

US EPA ARCHIVE DOCUMENT

451327-01

**Analytical Protocol  
(Amended)  
Clean-Up Procedures for Low Moisture & High Matrix Commodities**

**QUANTITATION OF FENPYROXIMATE RESIDUES  
IN  
RAW AGRICULTURAL AND PROCESSED COMMODITIES**

Author

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Prepared for

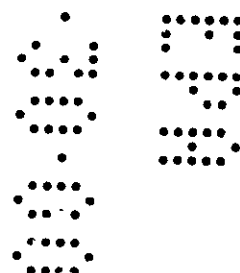
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Date Prepared

May 26, 2000



**GOOD LABORATORY PRACTICE STATEMENT**

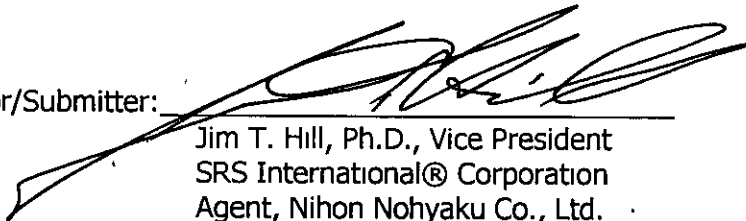
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This Study, an Analytical Protocol, is not subject to nor conducted under the  
Good Laboratory Practice requirements of 40 CFR Part 160

Sponsor/Submitter:



Jim T. Hill, Ph.D., Vice President  
SRS International® Corporation  
Agent, Nihon Nohyaku Co., Ltd.

Date:

May 26, 2000

**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

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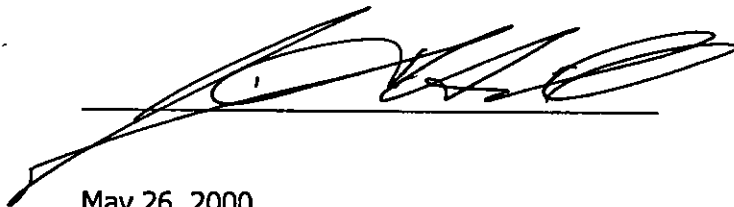
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Sections 10(d)(1)(A), (B), or (C).

Company: Nihon Nohyaku Co., Ltd.

Company Agent: Jim T. Hill, Ph.D., Vice President

SRS International® International

Signature:

A handwritten signature in black ink, appearing to be "Jim T. Hill", written over a horizontal line.

Date:

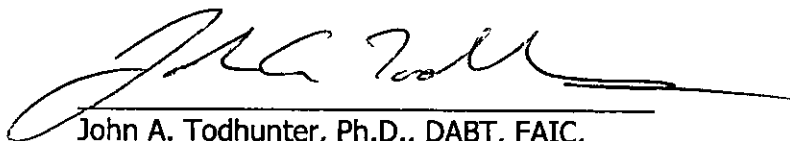
May 26, 2000

**Protocol Approval**

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John A. Todhunter, Ph.D., DABT, FAIC,  
Sponsor's Representative  
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Date: May 26, 2000

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## Reference:

**MRID # 540972-01**

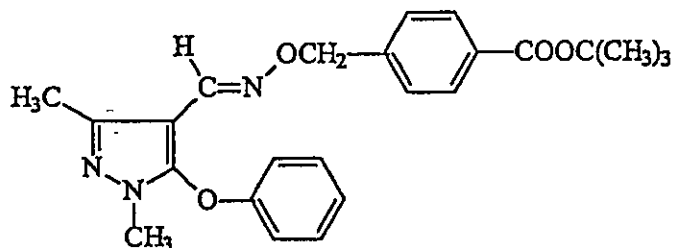
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**"Analytical Protocol, Quantitation of Fenpyroximate Residues in Raw Agricultural and Processed Commodities"** – Author, Jim T. Hill, Ph.D., SRS international® Corporation, March 30, 2000

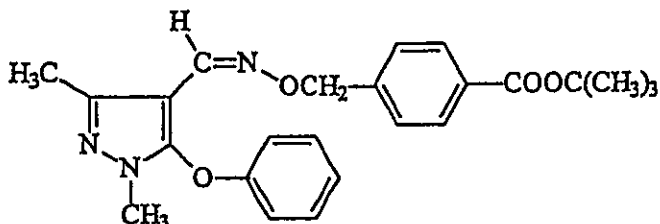
## 1. INTRODUCTION

Nihon Nohyaku produces Fenpyroximate, CAS Registry No. 134098-61-6, Benzoic acid, 4- [[(E) - (1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]-, 1,1dimethylethyl ester. The major degradate of fenpyroximate is M-1, its geometric (Z) isomer Benzoic acid, 4- [[(Z) - (1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]-, 1,1dimethylethyl ester. In support of an U.S. Import Tolerance Petition on grapes and hops for fenpyroximate, the magnitude of the residues of fenpyroximate in/on raw and processed agricultural commodities was determined. These agricultural commodities included apples, citrus, cotton, hops and vine (grapes).

The chemical structure of fenpyroximate is given below:



The chemical structure of M-1 is given below (Z-isomer):



Treated and control raw agricultural commodities as well as the processed agricultural commodities should be stored and shipped frozen for analysis. Untreated control and treated samples of each commodity and two fortified control samples will be extracted and analyzed using gas chromatography-nitrogen-phosphorous detector (GC-NPD).

## 2. SAMPLE ANALYSIS

The analysis procedure for the extraction and quantitation of fenpyroximate and M-1 was based on DFG Method S19, ("Organochlorine, Organophosphorus, Nitrogen-Containing and Other Pesticides - Gas-chromatographic determination after cleanup by gel permeation chromatography and silica gel mini column chromatography", Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, edited by Hans-Peter Thier and Hans Zeumer, Volume 1, 1987). This generic multi-residue method was optimized for the determination of fenpyroximate and M-1 at a limit of detection of 0.02 µg/g with a limit of quantitation of 0.05 µg/g in hop and vine (grape).

## 2.1 Principle of the Method

The samples were extracted with acetone. Prior to extraction, the water content of samples was adjusted to constant water content as necessary. Extracts were cleaned using gel permeation chromatography (GPC) followed by silica gel solid phase extraction (SPE). Analytes were eluted from the GPC column by using 1:1 ethyl acetate:cyclohexane and from the SPE column using 95:5 toluene:acetone. Analysis is by gas chromatography, BD-1 capillary column – nitrogen/phosphorus flame detector. Residues were quantitated by comparing peak areas of the test samples with the peak areas of the calibration standard prepared from known analytical standards. A series of calibration standards were also analyzed to confirm the linear range of the method. The laboratory may chose to calculate the residues of fenpyroximate/M-1 in hop or vine using peak heights, dependent on equipment availability or operator preference.

This amended protocol does not modify the gas chromatography method as reported in the MRID # 450972-01.

There are two modifications to the sample preparation in this protocol (amended). The first is a more rigorous clean-up of the sample matrix for low moisture commodities such as hops, cotton and dried fruit or dried fruit pulp and for certain high matrix fruit such as citrus. The clean up also includes a rigorous cleaning of the gas chromatograph between each set of samples. The second is the calculation of the "total" Fenpyroximate. Isomerization between the M-1 (Z-isomer) and the parent, fenpyroximate, was noted in the certain low moisture commodities. This isomerization is not constant between crop matrices and varies some even in the same matrix. These modifications are described in this protocol (amended). For additional details of the gas chromatography procedures please refer to the reference: MRID # 540792-01.

These modifications to the clean-up procedures are adaptable to processed commodities of grapes (wine) and hops (beer) if necessary. These commodities (materials) do not appear to need the additional sample preparation.

The clean-up procedure has been adapted for hops, cotton gin trash/cottonseed and other low moisture commodities. Dried hops will be used to illustrate the additional extract clean-up procedure(s).

## 2.2 Apparatus •

Analytical Evaporator, Zymark TurboVap, Model 11

Analytical Evaporator, Zymark TurboVap, Model LV

Analytical Evaporator, Organomation N-Evap

Blender, Waring 7075B, Model 35BL33

Food Processor, Mr. Coffee™, model IDS-50 (mill type to course grind sample)

Homogenizer, Hobart, Model 84186 (course grind larger sample sizes)

Pipette, motorized, 1.0 mL, Rainin

Pipette, motorized, 100 µL, Rainin

Pipettes, manual, 10 µL to 500 µL

Pipettes, Pasteur

Syringe filter, Gelman Acrodisk (13 CR PTFE 0.45 µm)

Vacuum Pump, General Electric

Vacuum Manifold, Supelco

Gel Permeation Chromatography and Autovap, ABC, Model AS-2000

Vortex Mixer, Model G-560

Standard laboratory glassware

- Equivalent (or similar) apparatus may be substituted.

## 2.3 Materials, Reagents, and Solvents

**Fenpyroximate:** Benzoic acid, 4- [[[(E) – (1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]-, 1,1dimethylethyl ester, CAS Registry No. 134098-61-6. The analytical standard was obtained from Nihon Nohyaku Co., Ltd., Chuo-Ku Tokyo, Japan. The standard, purity 99.8% by HPLC, is stable when stored frozen, (-15°C) in the dark.

**M-1:** Benzoic acid, 4- [[[(Z) – (1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]-, 1,1dimethylethyl ester CAS Registry No., unknown. The analytical standard was obtained from Nihon Nohyaku Co., Ltd., Chuo-Ku Tokyo, Japan. The standard, purity 98.6% by HPLC, is stable when stored frozen, (-15°C) in the dark.

Acetone, Burdick and Jackson, HPLC grade

Ethyl acetate, Baker Analyzed, HPLC grade

Silica Gel, Mallinckrodt (equivalent to Merck 7734, grade 60, 0-230 mesh, 60A<sup>0</sup>)

Cyclohexane, J. T. Baker, HPLC grade

Celite 545, J. T. Baker, pesticide grade

Hexane, Burdick and Jackson brand, HPLC grade.

Isooctane, Burdick and Jackson brand or J. T. Baker, HPLC grade

Methylene chloride, Burdick and Jackson, HPLC grade

Sodium chloride, Baker, Analyzed

Sodium sulfate, Mallinckrodt, anhydrous, reagent grade, granular, 99%

Toluene, Burdick and Jackson brand, HPLC grade

Filters, A/E glass fiber, 76 mm

1: 1 Ethyl Acetate:Cyclohexane

65:35 Hexane:Toluene

95:5 Toluene:Acetone

80:20 Toluene:Acetone

- Equivalent grades of materials, reagents and solvents may be substituted.

## **2.4 Preparation of Standard Solutions**

### **2.4.1 Stock Standard Solutions - Fenpyroximate**

A 10 mg aliquot of fenpyroximate was weighed into a 10 mL volumetric flask and brought to mark with toluene and mixed. The concentration of fenpyroximate stock standard solution was approximately 1000 µg/mL when corrected for chemical purity, 99.8% of standard.

### **2.4.2 Stock Standard Solutions - M-1**

A 10 mg aliquot of M-1 was weighed into a 10 mL volumetric flask and brought to mark with toluene and mixed. The concentration of M-1 stock standard solution was approximately 1000 µg/mL when corrected for the chemical purity, 98.6% of the standard.

### **2.4.3 Fortification Standards**

A set of fortification standards of fenpyroximate plus M-1, with concentrations of 10, 100 and 1,000 µg/mL, were prepared. The 100 µg/mL fortification standard was prepared by diluting the appropriate amounts (~1000 µL) of the fenpyroximate and M-1 stock standard solutions to 10 mL with ethyl acetate. The 10 µg/mL fortification standard solution was prepared by diluting 1000 µL of the 100 µg/mL fortification standard to 10 mL with ethyl acetate.

### **2.4.4 Calibration Standards**

A 10 µg/mL calibration stock solution was prepared by diluting 1000 µL of the 100 µg/mL fortification standard to 10 mL using toluene:acetone (95:5). Serial dilutions of this solution were prepared in toluene:acetone (95:5) to yield calibration standards at 0.25, 0.50, and 1.00 µg/mL. Serial dilutions of the 1.0 µg/mL calibration standard were prepared in toluene:acetone (95:5) to yield calibration standards at 0.02 and 0.05 µg/mL. [Toluene may be used as an alternate diluent.]

## **2.5 Sample Processing**

A typical procedure for sample processing is described below.

### **2.5.1 Sample Preparation**

Process frozen dried hop cones by coarsely grinding with an appropriate food processor (grinder mill such as MR. Coffee). The hop cones should be reduced to a size no smaller than very coarse coffee, pepper or tea flakes. Ten to fifteen grams of hop cones can easily be processed in a coffee grinder, larger samples should use appropriately sized equipment. Process prior to extraction (and/or fortification where appropriate).

### 2.5.2 Extraction with Acetone

Sample material is extracted with acetone. Water is added to the acetone in an amount that takes full account of the natural water content of the sample so that during extraction the acetone:water ratio remains constant. The extract is saturated with sodium chloride and diluted with methylene chloride, resulting in separation of excess water.

1. For extraction purposes, weigh ~10 grams of processed dried hop cones into appropriate vessel for extraction (typically 250 mL polypropylene bottle). Fortify samples as required for determining method recovery.
2. Extract sample by shaking for 10 minutes with 100 mL acetone/water 4:1 (v:v).
3. Filter sample extract, decant liquid, by vacuum filtration through Whitman #4 filter paper (typically 11 cm in Büchner funnel). Collect filtrate in vacuum flask (typically 500 mL).
4. Repeat extraction procedure once with 75 mL acetone/water 4:1 (v:v). Add ~10 grams Celite to extract then shake briefly and filter. Rinse extraction bottle with 2 x 25 mL acetone/water 4:1 (v:v) and transfer to filter cake, combining filtrates in vacuum flask.
5. Transfer entire volume of sample filtrate to separatory funnel (typically 500 mL). Rinse filter flask with 2 x ~10 mL acetone and combine rinses with filtrate in separatory funnel.
6. Add an excess amount of sodium chloride (~20 grams) to filtrate.

### 2.5.3 Liquid/Liquid Partition:

1. Add 100 mL dichloromethane (DCM) to separatory funnel and shake for ~2 minutes. Allow phases to separate completely (~10 minutes) before discarding lower, aqueous layer.
2. Add ~10 grams anhydrous sodium sulfate to separatory funnel and shake for ~2 minute.
3. To dry DCM fraction, pass DCM fraction through an ~1 inch bed of anhydrous sodium sulfate. Collect the dried DCM fraction in an appropriate flask. Rinse separatory funnel with 2 x ~20 mL ethyl acetate and combine rinses with dried organic fraction after passing ethyl acetate rinses through anhydrous sodium sulfate bed.
4. Concentrate DCM fraction to dryness by rotary evaporation under vacuum at ambient temperature. (To facilitate reconstitution of sample in 8 mL, it is advised that the extract concentration proceeds in a step-wise fashion using a relatively small flask, 500 mL or less).
5. Transfer sample to centrifuge tube, wash flask with 2 x 5 mL ethyl acetate, concentrate washes under gentle stream of nitrogen (ambient temperature). Repeat step 5 twice.
6. Reconstitute residue in 4 mL ethyl acetate followed by 4 mL cyclohexane.

#### 2.5.4 Gel Permeation Chromatography

Typically, GPC was conducted using the conditions described below.

Column:	Enviro-Beads S-X3, 60 gm, ~ 50 cm height Glass column – 25 mm (id) X 70 cm
Mobile Phase:	1:1 ethyl acetate:cyclohexane
Injection Volume:	5 mL
Dump Time:	23 minutes
Collect Time:	8 minutes
Wash Time:	10 minutes
Sampling loop:	5 mL
Flow rate:	5 mL/minute

The fenpyroximate elute/solvent location was determined by adding a standard solution of fenpyroximate and M-1 (in ~5 mL of 1:1 ethyl acetate:cyclohexane) to the column and eluting as described above. Elution of fenpyroximate was determined using an UV flow-through detector at 254 nm. An additional volume of 1-mL to each side of the elution peak was added to determine the fraction collected for the analysis.

1. Load sample onto GPC using glass syringe fitted with a Gelman Acrodisk filter (13 CR PTFE 0.45  $\mu$ m). (It is important that GPC sample loop volume is exactly 5 mL and that sample transfer-line is not more than 2 mL in volume. These volumes assure a sample load of 5 mL i.e. 5/8 of total sample volume).
2. After GPC cleanup, the sample was concentrated to approximately 1 mL with the Zymark TurboVap. The sample was transferred to a 10-mL conical tube and the TurboVap tube rinsed with 1 mL 1:1 ethyl acetate:cyclohexane and 5 mL isooctane and concentrated to dryness by under vacuum at approx. 35°C.
3. Reconstitute sample in 3 mL toluene/acetone (8:2, v:v). Sonicate for 5-10 seconds.

### 2.5.5 Silica Gel Minicolumn Chromatography

The entire sample was loaded onto a silica gel minicolumn. The column was rinsed with hexane, 65:35 hexane:toluene and toluene. The column was then eluted with 95:5 toluene:acetone. The concentrate (~1 mL) was transferred in toluene for analysis by gas chromatography – nitrogen-phosphorous detection (GC-NPD). Samples outside the range of the standard curve were diluted to appropriate levels using 95:5 toluene:acetone as the solvent.

The fenpyroximate elute/solvent location was determined by adding a standard solution of fenpyroximate and M-1 in toluene to the column and eluting as described above. Elution of fenpyroximate was determined using an UV flow-through detector at 254 nm. An additional volume of 1-mL to each side of the elution peak was added to determine the fraction collected for the analysis.

1. Prepare silica gel chromatographic column with ~10 grams activated silica gel (60-200 mesh) top with ~1 – 2 cm of anhydrous sodium sulfate. Activate silica gel by removing water (4 hours at ~ 130°C). IMPORTANT: Column should have a 15 mm I.D., and should be wet-packed with a silica slurry. Before loading sample, elute excess solvent to top of sodium sulfate packing and discard.
2. Transfer sample to silica gel column. Allow sample to migrate into silica to top of packing before proceeding. Rinse concentration flask with hexane/acetone (8:2, v:v) 3 x 3 mL and add to column. Discard eluant.
3. Add 10 mL hexane/acetone (8:2, v:v). Discard eluant.
4. Elute analyte(s) with an additional 40 mL hexane/acetone (8:2, v:v). Collect in 125 mL conc. flask.
5. Concentrate to ~1-2 mL by rotary evaporation at ~35°C. Quantitatively transfer concentrate to test tube with hexane/acetone (8:2, v:v) rinses of 125 mL conc. flask, then continue concentrating to dryness under a stream of nitrogen gas.
6. Reconstitute sample in 1 mL toluene (dilute as needed). Analyze by GC/NPD.

Note: A commercially prepared SPE cartridge from chromatographic suppliers such as Supelco or Varian may used to replace the preparation of the activated silica gel column.

## 2.6 Sample Analysis

The gas chromatographic method described below is the same as DFG - S19 and that reported in MRID # 540972-01. Minor adjustment in the oven temperatures (ramping) and other chromatography parameters, at the operator's discretion, may be made to compensate for variations in commodities, matrix extract, equipment, columns, peak definition or personal preference. (However, the operator is cautioned to rigorously clean the injection system of the chromatograph after each set of samples is analyzed.) This port cleaning should eliminate the build-up of any matrix residue from all extract samples.

The calibration/calculation of the fenpyroximate was altered based on two observations, first the isomerization of fenpyroximate to its Z-isomer (M-1) and M-1's re-isomerization to the parent. The second observation was the significant enhancement of the fenpyroximate recovery in certain commodities' matrix. To compensate for the fenpyroximate – M-1 isomerization and the noted fenpyroximate recovery enhancement "total" fenpyroximate should be calculated by combining the peak values (area or height) for both fenpyroximate and M-1.

### 2.6.1 Gas Chromatography - Nitrogen-Phosphorous Detector

Typical GC/NPD conditions for the determination of "total" fenpyroximate (parent and M-1) in hop/vine:

#### Apparatus

Gas Chromatograph: VARIAN 3400 with autosampler

Detector: Alkali flame ionization detector (N-FID),

Data Systems: HP 3365 Chemstation

Column: DB-1 bonded phase, 15 m x 0.53 mm i.d. -  
0.15  $\mu$ m film thickness, J&W (used for method  
validation -hop, vine & beer, wine)

Inlet Liner: silanized dual tapered, splitless

The gas chromatograph injection port should be thoroughly cleaned between sample sets due to matrix build-up in the injection port. Certain matrix extracts may also bleed onto the front end of the column.

1. Dependent on the type of inlet liner it should be replaced or if not appropriate, the liner must be cleaned by thorough washing with solvents.
2. The injector port should be scrubbed with two solvents (cotton tipped swab) such as methanol and hexane until no residue is evident.

3. If there is evidence of "matrix" interference after cleaning, the front of the column may be clipped (10 – 15 cm) until the column performance deteriorates to an unacceptable level.

### **Operating Conditions**

Oven Temperature:	150°C – hold 2 min 150-240°C at 10°C/min 240°C - hold 10 min
Injector Temperature:	230°C
Detector Temperature:	250°C
flow rate:	air 175 mL/min hydrogen 4.5 mL/min
Carrier Gas:	Helium
flow rate:	approximately 30 mL/min
Injection Mode:	splitless
Injection Liner:	Silanized dual taper
Injector Purge Delay:	0.75 minutes
Septum Purge:	2.0 mL/minute
Injection Volume:	5 µL (hop) 1 µL (vine)

1. The above operating conditions were used for method validation and residue quantitation of hop and vine.
2. Minor changes to the operating conditions may be made during sample analyses to minimize any matrix interferences and to compensate for minor column size difference.
3. Typically, the autosampler was loaded with vials containing solvent/reagent blank (95:5 toluene acetone), a minimum of four calibration standards, plus analytical sample extracts (control, treated & recovery). A 2.5 µg/mL standard, for hops or 0.5 µg/mL, for vine, was typically injected before and after each set (typically four) of analytical samples.

- Alternate equivalent analytical instruments may be used for this chromatographic analysis.

## 2.6.2 Calibration and Calculations

### Calibration

Typical calibration curves obtained by plotting the peak response versus concentration ( $\mu\text{g/mL}$ ) of fenpyroximate or M-1 separately injected are presented in Figure 1 of MRID # 540972-01. The detector response was linear over the range of 0.05 to 2.50  $\mu\text{g/mL}$  of fenpyroximate or M-1. Calibration curves were used only to determine instrument linearity. Quantitation was accomplished utilizing the average response factor for the two standards nearest to the expected value, which bracketed the sample.

### Sample Calculation

The following equations were used to calculate the analyte concentration in the extract, final concentration in the sample, and expected concentration and percent recovery in fortified samples.

Concentration in Extract ( $\mu\text{g/mL}$ ) = (Peak Response x Response Factor)

where response factor is calculated from:

$$\text{Response Factor} = \frac{\text{Concentration of Standard Mixture (} \mu\text{g/mL)}}{\text{Area of peaks for Fenpyroximate/M-1 Standard}}$$

$$\text{Final Concentration (} \mu\text{g/g)} = \frac{C \times V_f \times S}{W}$$

where:

C = concentration in extract ( $\mu\text{g/mL}$ )

$V_f$  = final volume of the sample extract (mL)

S = extract split factor (3)

W = weight of the sample (g)

$$\text{Expected Concentration } (\mu\text{g/mL}) = \frac{A \times B \times D}{C \times E}$$

where

A = concentration of spike solution ( $\mu\text{g/mL}$ )

B = amount of spike solution (mL)

C = final volume

D = concentration volume (8 or 15 mL)

E = GPC sample loop size (5 mL)

$$\% \text{ Recovery } (\mu\text{g/mL}) = \frac{\text{concentration in extract}}{\text{expected concentration}} \times 100$$

## 2.7 Method Validation

Unfortified hops, dried cones (control) prepared in triplicate to represent zero ( $0.0 \mu\text{g/g}$ ) concentration of "total" fenpyroximate. Triplicate control hops spiked with fenpyroximate/M-1 standard mixtures to  $0.05 \mu\text{g/g}$  (low fortifications),  $0.25$ ,  $0.50 \mu\text{g/g}$ , or  $1.00 \mu\text{g/g}$  (mid fortifications), and  $2.00 \mu\text{g/g}$  (high fortification).

1. Check standards were acceptable if total peak areas were  $\pm 20\%$  of the calibration curves of the values.
2. Recoveries were acceptable if recovery calculated was  $\pm 20\%$  of the expected concentration.
3. Calculated values were not adjusted for recovery.
4. Peak areas were not adjusted for "control" peak values.

### 3. DISCUSSION

Samples of hops (dried cones), cotton (seed & gin trash), vine (grapes, raisins) have processed, extracted and analyzed by GC-NPD using the methods described in this amendment. Recoveries for fenpyroximate in hop were ~114% and in grape were ~85%. The recoveries obtained for M-1 in hop were ~79% and in grape ~90%. No fenpyroximate or M-1 was detected in the unfortified control samples. Recovery estimated using Fenpyroximate/M-1 calibration standards was ~115% in both grapes and hops. Standard chromatograms of fenpyroximate and M-1 are shown in Figure 1 and Figure 2 using calibration standards of 0.5 & 1.00 µg/mL. Residues of M-1 were not found at or above the method detection limit (0.02 µg/g) in most of the treated commodity extracts. Extracts with higher concentration of fenpyroximate, such as treated hop, were analyzed twice. Once as a "concentrated" extract to determine if M-1 was present and the second time as a diluted extract to quantitate the fenpyroximate. The initial extract (GC sample) was diluted to the estimated concentration of fenpyroximate/M-1 used in the calibration standards.

Typical chromatograms of the analytical standards for fenpyroximate and M-1, spiked untreated and treated crops as well as selected processed products are illustrated by the Figures included in the original protocol, MRID # 540972-01.

The gas chromatographic conditions, peak height quantitation, was validated specifically in hops and vine (grapes). Note both Hewlett Packard and Varian gas chromatographs have been used for these gas chromatographic procedures.

- Alternate equivalent analytical instruments may be used for this chromatographic analysis.

The estimated time for the analysis for a set (12 to 18 samples) of treated commodities is a maximum of four man-days each for two technicians working concurrently. The extraction and clean up of the crop samples plus the preparation of standards requires about eight man-days. The GC analysis is usually completed overnight; the following day is used for compilation, calculations and review of the analysis (single technician).

Limit of detection is defined as the lower standard used (quantitated) in the preparation of the calibration curves.

Limit of Detection:

Grapes	0.02 µg/g	Hops	0.02 µg/g	Cotton	0.02 µg/g
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Limit of quantitation is defined as the lowest fortification level yielding acceptable recoveries (80% to 120%) at analysis.

Limit of Quantitation:

Grapes	0.05 µg/g	Hops	0.05 µg/g	Cotton	0.05 µg/g
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It was apparent in using the first protocol procedure that there was a difference in instrument response for fenpyroximate in low moisture crops, dried fruit and high pulp content extracts (fruit matrix) *versus* extracts or standards that are predominantly liquid/solvent. This "matrix effect" enhanced the peak response for fenpyroximate and therefore gave unusually high recoveries for many fortified samples (<250%). A much smaller "matrix" effect was also observed for M-1.

The matrix effect appears to be caused by generic overloading of the chromatographic column apparently due to additional non-detectable materials extracted from the crop matrix. The matrix effect was not noticeable for the juices, ciders, or beer, but was usually evident in fruit pulp, whole fruit (citrus and apple) and dried fruit and hops. Based on the assumption that the matrix effect is caused by high concentrations of non-interfering compounds and/or non-detectable co-eluting compounds, significant changes were made to the processing of these materials. Minor changes were made to the gas chromatographic temperature program in an attempt to move the co-eluting peaks. The latter changes broadened all peaks slightly and reduced but did not eliminate the matrix effect. Additionally, sample extracts were also diluted as much as possible to make them as "solvent-like" as possible and minimize the amount of fruit-matrix injected.

The order in which extracts were analyzed was important. Extracts were analyzed in increasing order of fruit-matrix concentration. The calibration standards were injected, followed by the diluted treated and fortified extracts, and then the more concentrated extracts. Quantitation standards (similar values to expected concentrations) bracketed each set of extracted samples (usually ~ 4 samples). Standards bracketed at a minimum each dilution level, to minimize detector response drift while analyzing sets of extracts. Quantitation utilized averaged response factors from these standards. Minor peaks were observed near the retention times of M-1 and fenpyroximate in chromatograms from control low moisture extracts and control fruit extracts. These peaks did not appear to affect the quantitation of the treated samples. However, analyte recoveries from fortified samples not were corrected for the contribution of the interfering compounds.

Based on discussions with Agency and Contract Laboratory analytical chemists the gas chromatographic method appeared acceptable however, this method was sensitive to the amount of matrix materials that was carried through the clean-up procedures. The amended processing/clean-up procedures were developed following suggestions by the Agency and use of sample preparation modifications validated by Contract Laboratory personnel.

These modification include changes in the processing of low moisture samples, change to the extraction method (not the materials), increasing the size of the GPC and SPE columns and adding the rigorous cleaning of the gas chromatography system.

1. The sample size was reduced to ~10 grams, from ~20 grams without apparent significant loss in sensitivity. It appears even smaller sample sizes might be used but a corresponding loss in sensitivity would be expected.

2. Processing of low moisture samples was modified to include the coarse grinding of the hop sample instead of grinding to a fine powder in a blender. This modification did not appear reduce the recovery values.
3. The extraction proceed was modified to extraction by shaking rather than blending with the acetone as in the initial protocol. The color in the acetone/dichloromethane extract was reduced when the dried samples were coarse ground and shaken. These two changes appear to have reduced the amount of materials extracted from low moisture commodities.
4. The changes to the clean-up procedure were to increase the size of the "clean-up" columns.. Slight modifications to the elution volumes were made to accommodate the elution peak for fenpyroximate but not to the solvent systems.
5. Enhanced recoveries in high moisture content samples were infrequently noted after analysis of a large number of samples. The rigorous cleaning of the gas chromatograph injection system often controlled this enhancement without the inclusion of the expanded sample processing. Two contract laboratories have used this method on high moisture commodities. The laboratories also modified the column length to 30 meters to allow for the clipping of the front of the column. This modification did not cause significant change in separation. Gas flow rates changes were the only other reported modification to the GC method. However, additional clean-up appears to be always required for high matrix/low moisture samples.

The calibration and calculation were modified to accommodate the recognized isomerization between the parent, fenpyroximate and its Z-isomer, M-1. A small amount of parent to M-1 isomerization was noted in the field studies where the "major" metabolite was M-1. The amount of this isomerization was usually a few percentage. Significant isomerization from M-1 to parent was noted in several studies including hops storage stability and other dried commodities analysis. Up to 40% conversion was noted in the storage stability study enhancing the recovery of fenpyroximate in these reports. However, due to the variability of the isomerization between matrices the quantitation of individual compounds (fenpyroximate & M-1) could not be validated. The calibration/calculation of the "total" fenpyroximate, the sum of the fenpyroximate and its Z-isomer (M-1), has also been validated in processed cotton feed products and vine (fruit and processed).

#### **4. ARCHIVAL OF STUDY RECORDS**

All data and observations were recorded on data sheets or in notebooks, which will be provided to the Sponsor along with the final report. Verified copies of all notebooks and a copy of the final report will be archived the test laboratory. All unused test substance and any unused test sample will be properly disposed after the study final report is issued.

Standard:

M-1  
fenpyroximate 0.503  $\mu\text{g/ml}$   
0.505  $\mu\text{g/ml}$

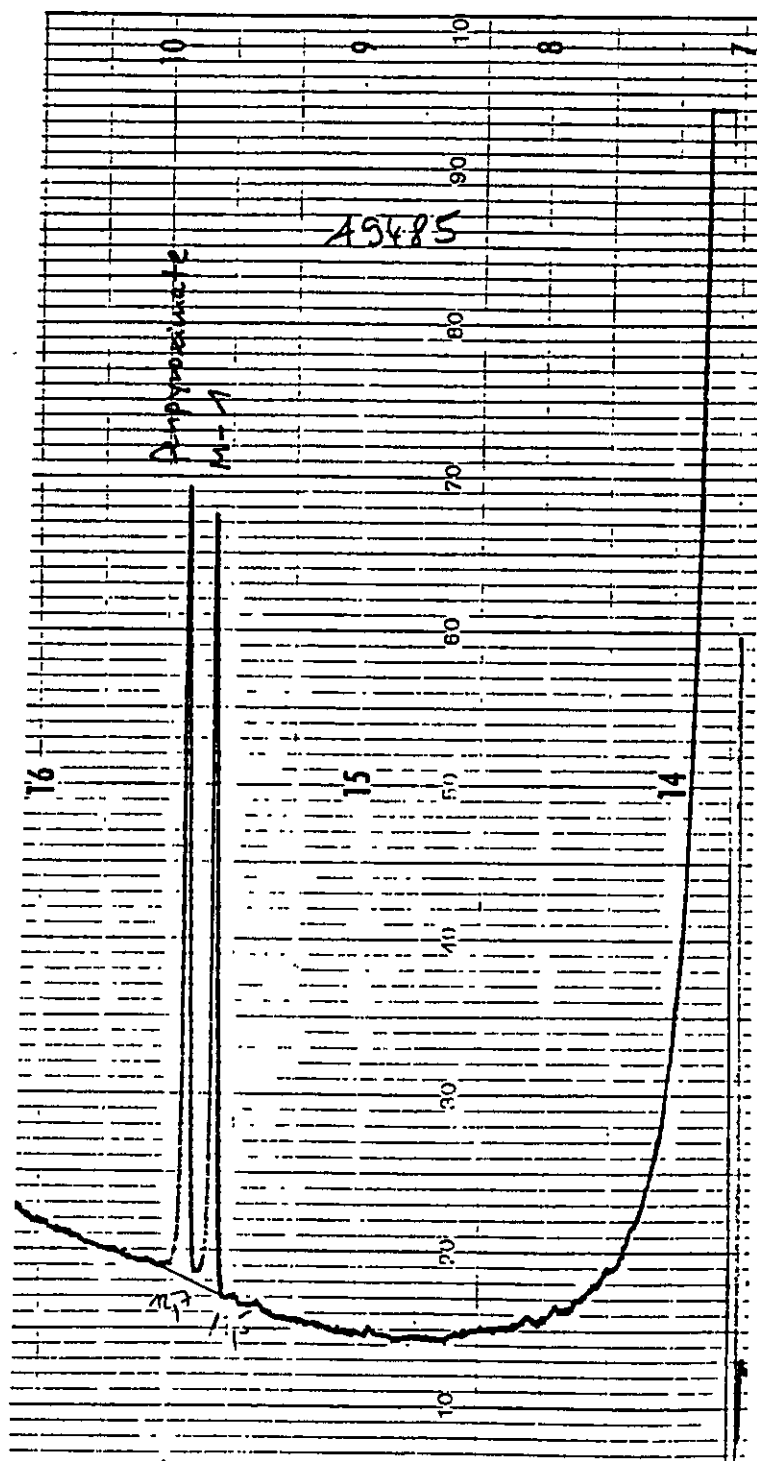


Figure 1. Fenpyroximate & M-1 Analytical Standard (0.5  $\mu\text{g/mL}$ )

External standard solution of  
 Fenpyroximate 1.20  $\mu\text{g/ml}$  and  
 M-1 1.21  $\mu\text{g/ml}$

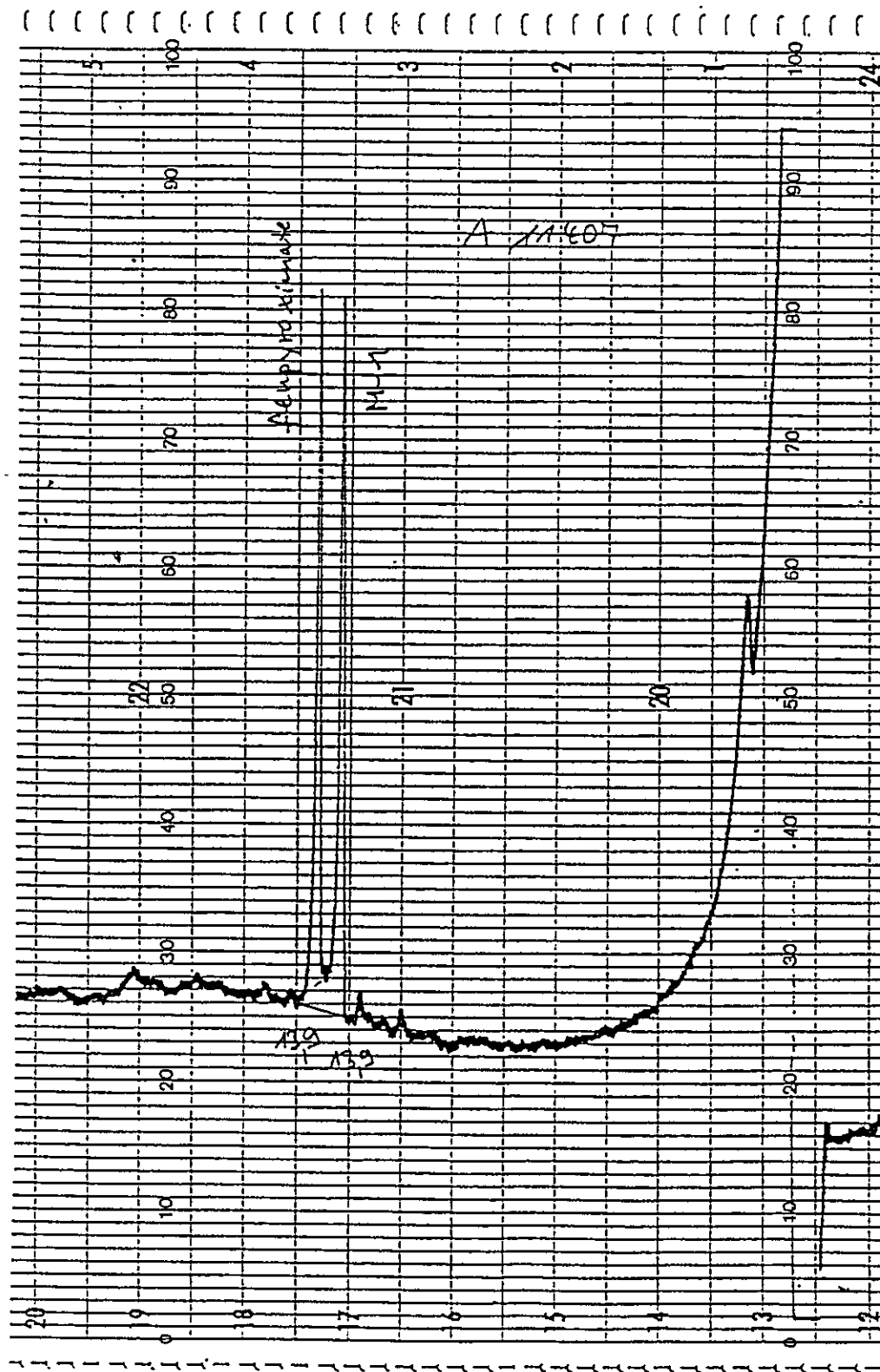


Figure 2. Fenpyroximate & M-1 Analytical Standard (1.0  $\mu\text{g/mL}$ )

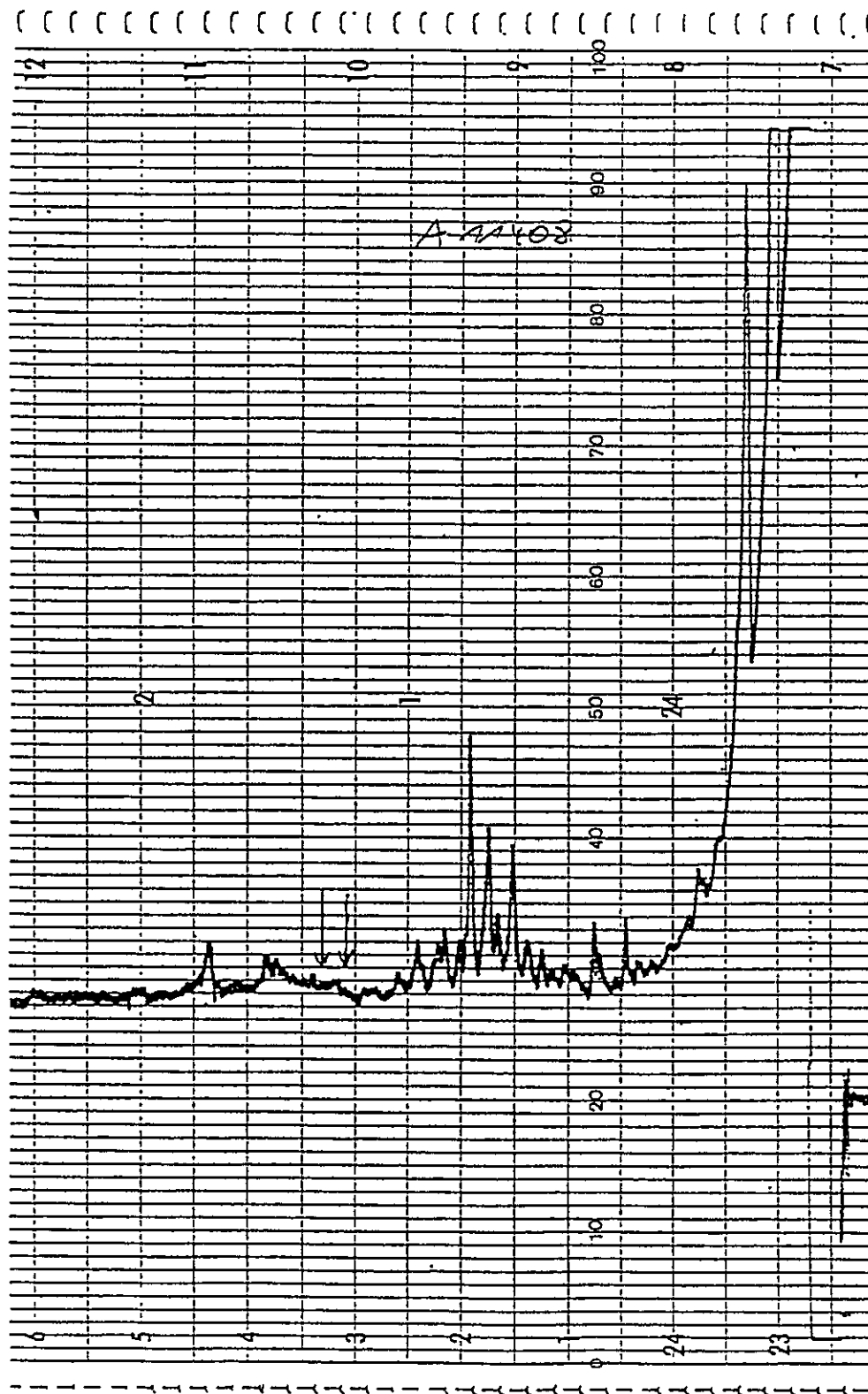


Figure 3. Typical Dried Hop Cone Chromatogram (Control)

Dried cones 804 0302 day 21 untreated  
spiked with M-1 1.0 mg/kg  
fenpyroximate 1.0 mg/kg

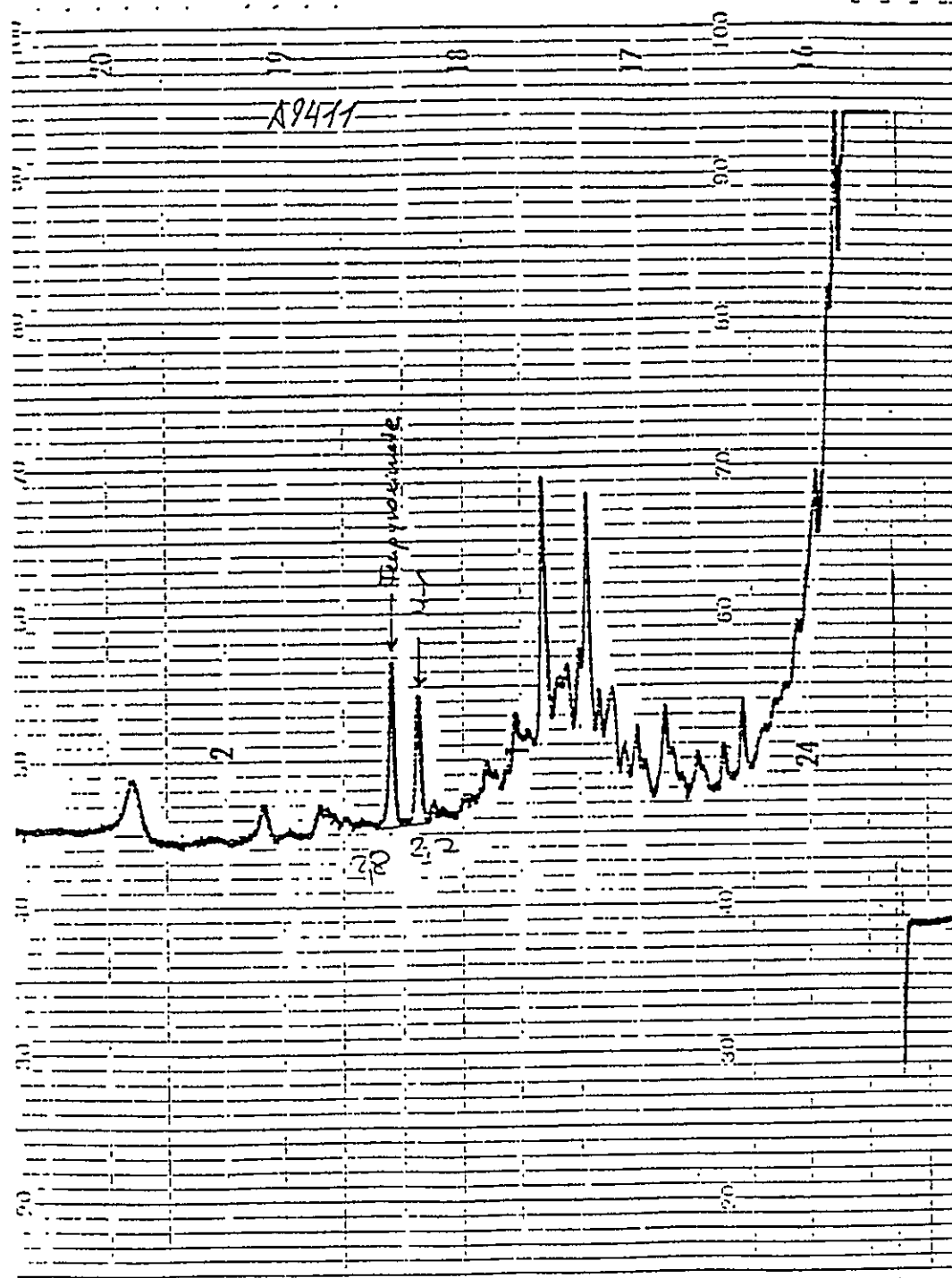


Figure 4. Dried Hop Cones Spiked with Fenpyroximate & M-1 (1.0  $\mu\text{g/mL}$ )

Dried cones 804 0302 day 21 treated

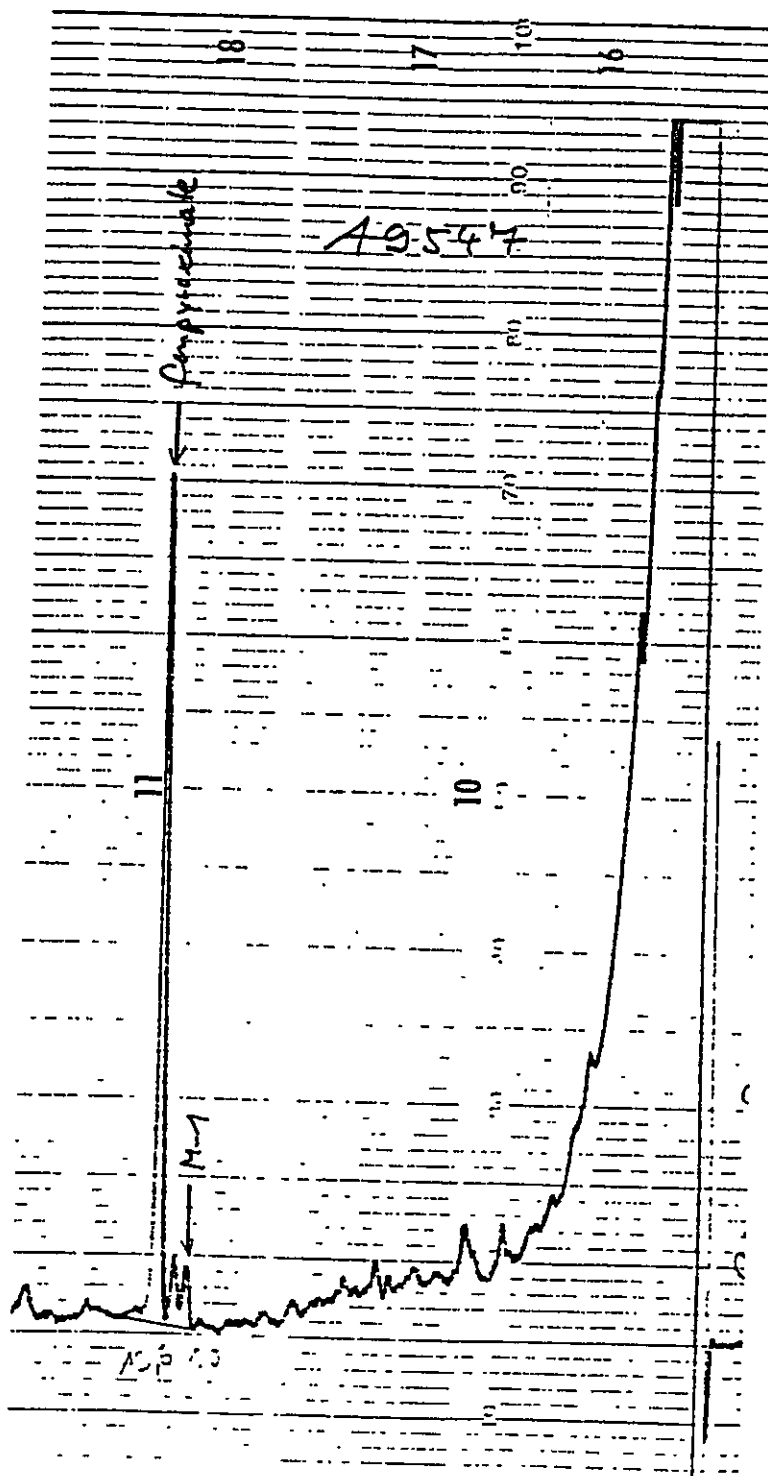


Figure 5. Typical Dried Hop Cones Treated (21 days, at 2X [15 Kg/ha], single treatment)

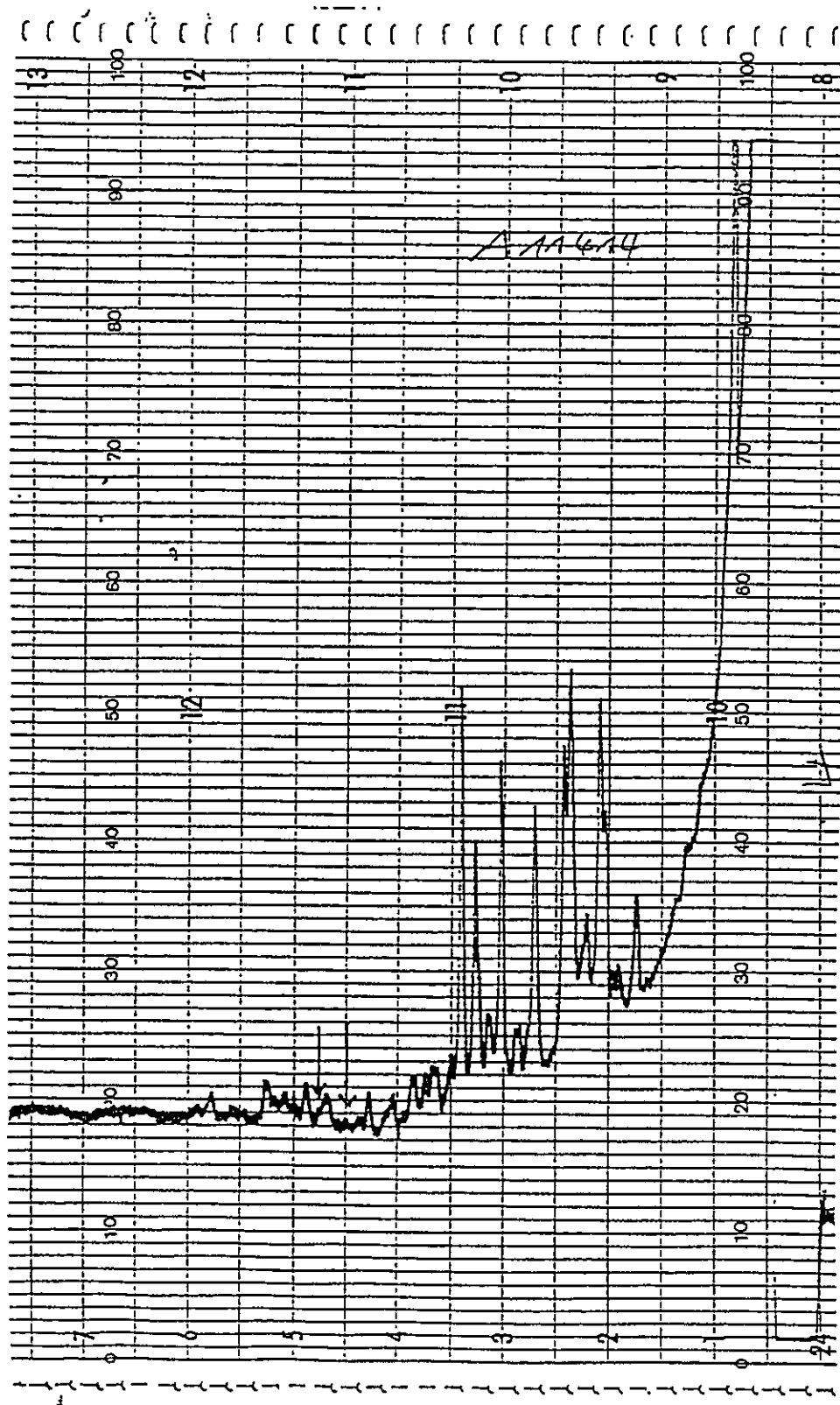


Figure 6. Dried Hop Cones Storage Stability (Control, 12 months)

Untreated sample of dried cones  
storage stability Fenpyroximate 9.60 mg/kg, 12 months

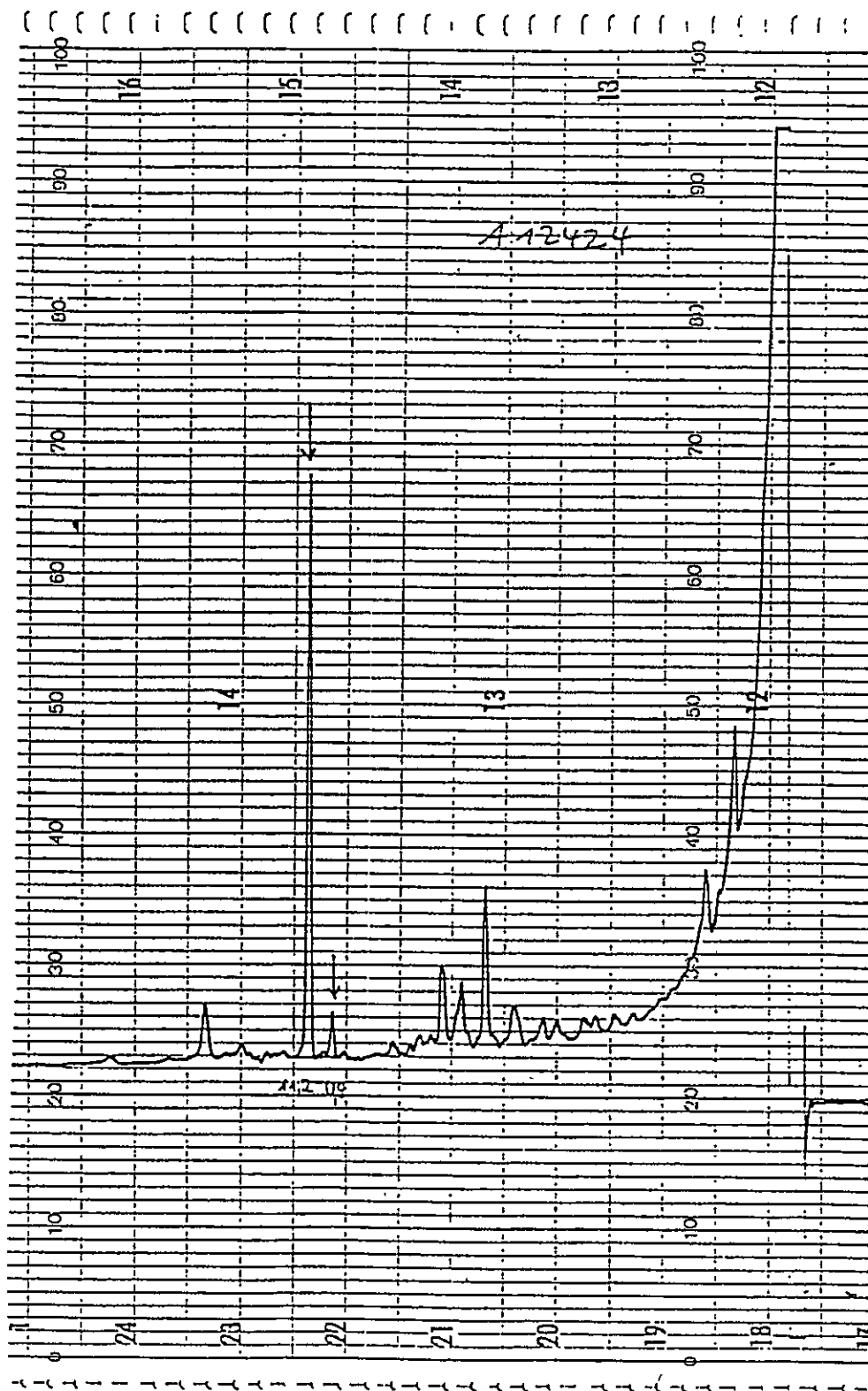


Figure 7. Dried Hop Cones Storage Stability (Spiked Fenpyroximate - 10 mg/Kg, 12 months)

Untreated sample of dried cones  
storage stability M-1 9.68 mg/kg, 12 months

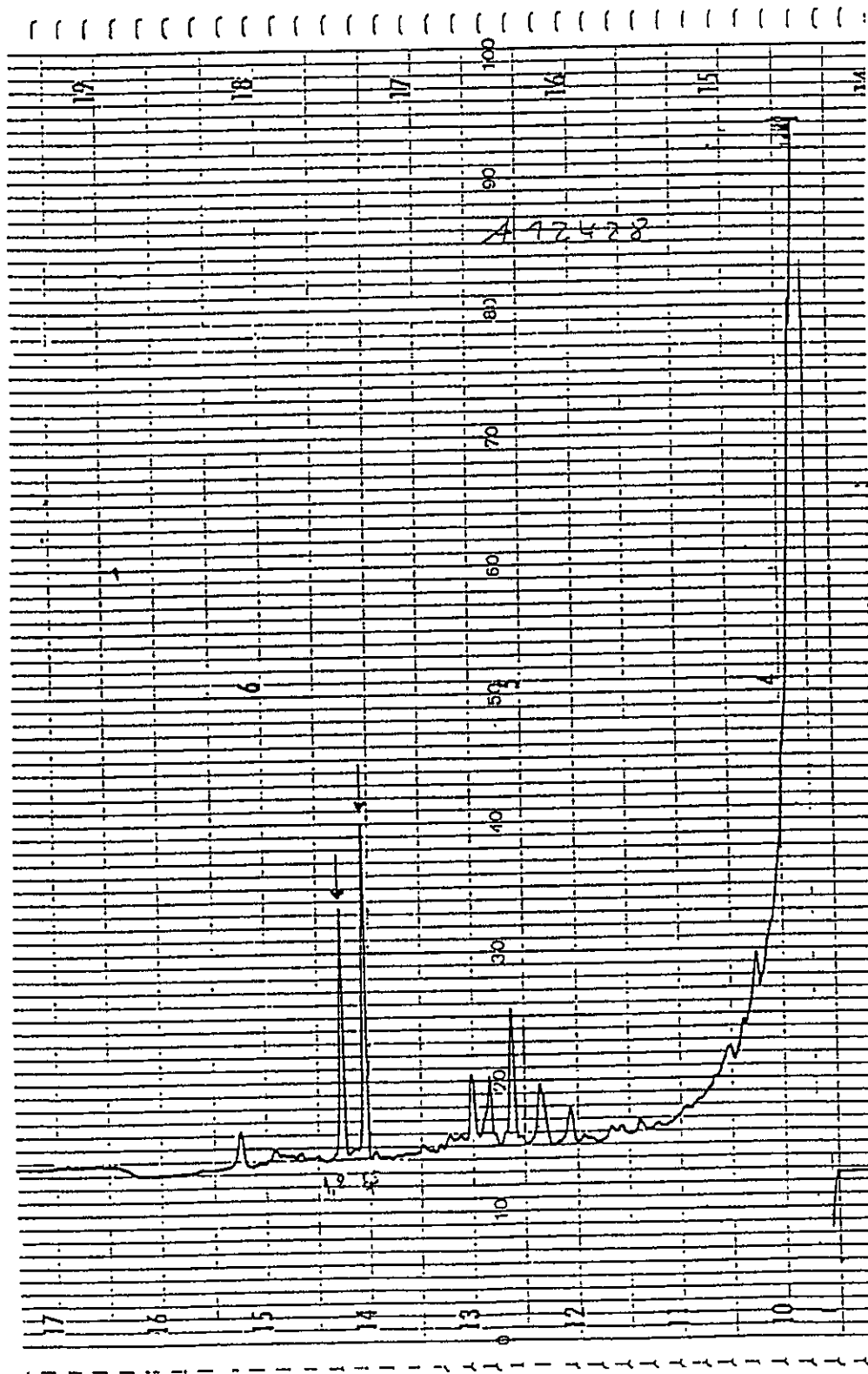


Figure 8. Dried Hop Cones Storage Stability (Spiked M-1, Z-isomer - 10 mg/Kg, 12 months)